

## **Remarks**

### **The Amendments**

The sequence listing has been amended to add the newly assigned filing date and serial number of the application at numeric identifiers <140> and <141>. The sequence listing has also been amended to delete SEQ ID NOs. The deleted sequences include SEQ ID NOs: 5-8, SEQ ID NOs: 11-14, and SEQ ID NOs: 22-25 which are duplicates of SEQ ID NOs: 1-4; SEQ ID NOs: 15 and 25 which are duplicates of SEQ ID NO: 5; SEQ ID NOs: 16, 17, 27 which are duplicates of SEQ ID NO: 6; and SEQ ID NO: 28 which is a duplicate of SEQ ID NO: 8. The sequence listing has also been amended at SEQ ID NO: 10, which was originally filed claimed SEQ ID NO: 21, to explain that the n's present in the nucleotide sequence can be a "g, a, t, or c" at numeric identifiers <220> - <223>. A copy of the sequence listing in a computer readable format is also enclosed. I believe that the content of the paper and computer readable format copy of the sequence listing are identical. The sequence listing introduces no new matter.

The specification has also been amended to insert sequence identifiers and to correct inadvertent clerical errors.

Claims 1, 3, and 4 have been amended to recite the correct sequence identifiers for the recited nucleotide sequences.

Claims 16 and 30 have been amended to insert sequence identifiers immediately following each of the recited nucleotide sequences.

None of these amendments introduces new matter.

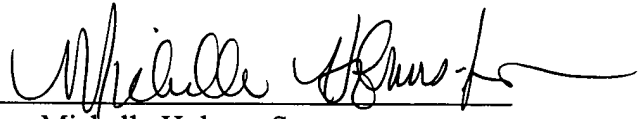
### **Sequence Listing Compliance**

The sequence listing was indicated as not compliant with the requirements of 37 CFR 1.822 and/or 1.832 because it contained a nucleotide sequence that contained n's and no

explanation was given as to what the n's of the sequence represent. The sequence listing has been amended at SEQ ID NO: 10, which was originally filed claimed SEQ ID NO: 21, to explain that the n's represent "g, a, t, or c" at numeric identifiers <220> - <223>.

Respectfully submitted,

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## Appendix 1. Marked Up Version of the Specification and Claims to Show Changes Made

For all amendments: underlining indicates insertions and striking through indicates deletions. Square brackets were present in the specification and claims of the application as filed and do not indicate deletions.

### The Claims

1. (Amended) A nucleotide sequence encoding a WT1 antisense regulatory region comprising at least a portion of the sequence shown in ~~SEQ.1~~ SEQ ID NO: 8 or at least a portion of a variant, due to base substitutions, deletions, and/or additions, of the sequence shown in ~~SEQ.1~~ SEQ ID NO: 8.

3. (Amended) A WT1 antisense regulatory region negative regulatory element (NRE) comprising at least a portion of the nucleotide sequence shown in ~~SEQ.2~~ SEQ ID NO: 9 or at least a portion of a variant, due to base substitutions, deletions, and/or additions, of the sequence shown in ~~SEQ.2~~ SEQ ID NO: 9.

4. (Amended) A WT1 antisense regulatory region NRE according to claim 3 wherein the NRE comprises the sequence shown in bold in ~~SEQ.2~~ SEQ ID NO: 9, or variants of such a sequence due to base substitutions, deletions and/or additions.

16. (Amended) A method according to claim 15 wherein the PCR assay system uses at least one of the following primers to amplify a region of nucleotide sequence:

Tf: 5'-GGGTGGAGAAGAAGGATATATTTAT-3'. (SEQ ID NO: 1)

Tr: 5'-TAAATATCAAATTAATTTCTCATCC-3'. (SEQ ID NO: 2)

TfN: 5'-GATATATTTATTTATTAGTTTTGGT-3' (SEQ ID NO: 3; nested primer).

Trn: 5'-AAACCCCTATAATTTACCCTCTTC-3' (SEQ ID NO: 4; nested primer).

30. (Amended) A method according to claim 29 wherein the RT-PCR uses the following primer pair

Primer 1: WT18 CTTAGCACTTTCTTCTTGGC (SEQ ID NO: 5)

Primer 2: WITKBF2 TTGCTCAGTGATTGACCAGG. (SEQ ID NO: 6)

### **The Specification**

The paragraph on page 6, lines 6-14.

The methylation state may be determined using a PCR-based assay system. Such a PCR-based assay system may involve the use of sodium-metabisulphite. This has the effect of converting all unmethylated cytosine residues to uracil residues. Preferably, the PCR reaction uses the following primers to amplify at least a portion of the WT1 antisense regulatory region:

Tf: 5'-GGGTGGAGAAGAAGGATATATTTAT-3'. (SEQ ID NO: 1)

Tr: 5'-TAAATATCAAATTAATTTCTCATCC-3'. (SEQ ID NO: 2)

TfN: 5'-GATATATTTATTTATTAGTTTTGGT-3' (SEQ ID NO: 3; nested primer).

TrN: 5'-AAACCCCTATAATTTACCCTCTTC-3' (SEQ ID NO: 4; nested primer).

The paragraph on page 6, lines 21-25.

The nested PCR reaction involves the following primers.

Tf: 5'-GGGTGGAGAAGAAGGATATATTTAT-3'. (SEQ ID NO: 1)

Tr: 5'-TAAATATCAAATTAATTTCTCATCC-3'. (SEQ ID NO: 2)

TfN: 5'-GATATATTTATTTATTAGTTTTGGT-3' (SEQ ID NO: 3; nested primer).

TrN: 5'-AAACCCCTATAATTTACCCTCTTC-3' (SEQ ID NO: 4; nested primer).

The paragraph on page 7, lines 16-20.

The RT-PCR may use two primers designed to anneal to a tumour-specific gene sequence on opposite sides of an allelic ~~polymorphism~~ polymorphism which introduces a restriction-site in one allele only. For example, in the case of WT, the RT-PCR may use the following primers:

Primer 1: WT18 [CTTAGCACTTTCTTCTTGGC] (SEQ ID NO: 5)

Primer 2: WITKBF2 [TTGCTCAGTGATTGACCAGG] (SEQ ID NO: 6)

The paragraph on page 8, lines 7-9.

Nucleotide sequences, and methods of disease diagnosis, detection and prognosis in accordance with the invention will now be described, by way of example only, with reference to accompanying Figures 1(A) to 3(B), and ~~SEQ-1~~ SEQ ID NO: 8 to ~~SEQ-3~~ SEQ ID NO: 10 in which;

The description on page 8, lines 24-30.

Figure 3(B) shows a southern blot of the antisense WT1 RNA RT-PCR products; and

~~SEQ-1~~ SEQ ID NO: 8 shows a nucleotide sequence of the WT1 ARR; and

~~SEQ-2~~ SEQ ID NO: 9 shows a nucleotide sequence of a negative regulatory element of a gene encoding WT-1; and

~~SEQ-3~~ SEQ ID NO: 10 shows the nucleotide sequence of a WT1 antisense region (Gessler, M & Bruns (1993) Genomics 17:499-501) with the RT-PCR primers shown as arrows and the exonic sequences indicated in bold.

The paragraph beginning on page 11, line 23 and ending on page 12, line 1.

Illustrative primers which may be used for methylation-specific PCR are shown below, and their ~~hybridisation~~ hybridization positions to the WT1 sequence are shown by arrows in Figure 2 for top-strand amplification. Allowing for C→T conversion, these are:

Tf: 5'-GGGTGGAGAAGAAGGATATATTTAT-3'. (SEQ ID NO: 1)

Tr: 5'-TAAATATCAAATTAATTTCTCATCC-3'. (SEQ ID NO: 2)

TfN: 5'-GATATATTTATTTATTAGTTTTGGT-3' (SEQ ID NO: 3; nested primer).

TrN: 5'-AAACCCCTATAATTTACCCTCTTC-3' (SEQ ID NO: 4; nested primer).

The paragraph on page 13, lines 18-25.

In order to determine whether the differential methylation of the *WT1* ARR/NRE is accompanied by allele specific expression of the *WT1* antisense RNA (*WT1-AS*), reverse transcription-PCR (RT-PCR) analysis was conducted on ~~foetal~~ fetal and normal kidney cells, and WT cells.

Primers either side of the antisense *WT1* RNA splice (see ~~SEQ 3~~ SEQ ID NO: 10 and Figure 3A) (Gessler, M., and Bruns (1993), *Genomics*, 17: 499-501, 1993) were used for RT-PCR:

Primer 1: WT18 [CTTAGCACTTTCTTCTTGGC] SEQ ID NO: 5

Primer 2: WITKBF2 [TTGCTCAGTGATTGACCAGG]. SEQ ID NO: 6

The paragraph on page 14, lines 7-14.

The PCR products obtained were digested by adding the restriction enzyme *MnII* directly to the PCR mix and incubating for 60 minutes at 37°C. The PCR products were then separated on 2% agarose gels and then alkali blotted onto Hybond N<sup>+</sup> membrane and ~~hybridised~~ hybridized with a <sup>32</sup>P-labelled antisense cDNA probe. The sequence of the probe is shown in bold between WT18 and WITKBP2 in ~~SEQ 3~~ SEQ ID NO: 10. The following primers were used as DNA controls:

Primer 1: WITKBF2 [TTGCTCAGTGATTGACCAGG] (SEQ ID NO: 6)

Primer 2: WITKBR2 [TTGGCTGGAAAGCTTGCAGC] (SEQ ID NO: 7)

Line 7 on page 15.

~~SEQ.1~~ SEQ ID NO: 8

Line 7 on page 16.

~~SEQ.2~~ SEQ ID NO: 9

Line 1 on page 17.

~~SEQ.3~~ SEQ ID NO: 10